# Effect of temperature history on the growth of *Listeria monocytogenes* Scott A at refrigeration temperatures \*

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The effect of pre-inoculation temperature on the subsequent growth of Listeria monocytogenes Scott A at  $5^{\circ}$ C was examined in microbiological medium, UHT milk, canned dog food, and raw ground beef (untreated and irradiation-sterilized). In microbiological medium, the duration of the lag phase was decreased when aerobic and anaerobic cultures were initially grown at  $\leq 28$  and  $\leq 13^{\circ}$ C, respectively. Subsequent exponential growth rates and maximum population densities of the  $5^{\circ}$ C cultures were not affected by temperature history. Differences in lag phase durations were also observed when L monocytogenes initially cultured at 19 and  $37^{\circ}$ C were grown at  $5^{\circ}$ C in UHT milk and some of the canned dog food varieties. Growth of L monocytogenes was not observed in either untreated or irradiation-sterilized raw ground beef. While temperature history can affect the growth kinetics of L monocytogenes at  $5^{\circ}$ C, it did not account for the lack of growth in raw meat, suggesting that there is an inhibitory condition or component in ground beef that is lost upon cooking.

Key words: Meat; Milk; Lag phase; Competition

## Introduction

While Listeria monocytogenes can grow in a variety of refrigerated foods including processed meat products, several teams of investigators have reported that the microorganism grows poorly, if at all, in refrigerated raw meat, particularly ground beef (Kahn et al. 1972, 1973, 1975; Gouet et al., 1978; Buchanan et al., 1987; Johnson et al., 1988a,b; Glass and Doyle, 1989; Shelef, 1989; Kaya and Schmidt, 1989). However, Kahn et al. (1972, 1973) demonstrated that L. monocytogenes grew on the surface of sterile lamb stored at 8°C. Gill and Reichel (1989) observed

growth on vacuum-packed beef strips at temperatures as low as 0°C, but did not detect growth when the meat was stored at <10°C in a CO<sub>2</sub>-enriched atmosphere. Grau and Vanderlinde (1988) reported that *L. monocytogenes* grew readily at 5.3°C on strips of beef, particularly on the adipose tissue. They hypothesized that other investigators did not observe growth at refrigeration temperatures due to the use of inocula grown at elevated temperatures (30–37°C), resulting in extremely extended lag phases when the microorganism was transfered to refrigerated meat. Grau and Vanderlinde (1988) used an inoculum grown at 10°C and observed a minimal lag phase. The objective of the current study was to test this hypothesis by examining the effect of temperature history on the growth kinetics of *L. monocytogenes* incubated at 5°C in microbiological medium, UHT milk, sterile cooked meat products, raw ground beef and irradiation-sterilized raw ground beef.

## **Materials and Methods**

## Microorganism

Listeria monocytogenes Scott A was used throughout the study. Stock cultures were maintained in Brain Heart Infusion Broth (Difco) at 4°C, and transferred monthly. Inocula were grown in 50 ml Tryptose Phosphate Broth (TPB) (Difco) in 250-ml Erlenmeyer flasks incubated for 144 h at 5°C, 96 h at 10°C and 13°C, 48 h at 19°C, or 24 h at 28°C, 37°C, and 42°C on a rotary shaker (150 rpm).

## Microbiological medium

The microorganism was cultured aerobically and anaerobically in TPB according to the method of Buchanan et al. (1989). TPB was adjusted to pH 6.75 with concentrated HCl, and 50-ml portions were transferred to either 250-ml Erlenmeyer flasks (aerobic cultures) or 250-ml trypsinizing flasks (anaerobic cultures). Erlenmeyer flasks were capped with foam plugs, and trypsinizing flasks were sealed with screw caps and rubber septa. All flasks were sterilized by autoclaving and then prechilled to 5°C. Starter cultures were diluted and apportioned to achieve an inoculum of approximately 10<sup>3</sup> cfu/ml in each flask. Immediately after inoculation, the trypsinizing flasks were flushed for 10 min with N<sub>2</sub> sterilized by filtration and sealed tightly to maintain anaerobiosis. All flasks were incubated at 5°C on a rotary shaker (150 rpm). At least two replicate cultures were studied for each temperature/ atmosphere combination. Periodically, 2.5-ml samples were removed from the Erlenmeyer and trypsinizing flasks using a pipette or hypodermic needle + syringe, respectively. Flasks were kept in an ice-water bath during sampling to ensure that the 5°C incubation temperature was maintained continuously. Samples were diluted appropriately with sterile 0.1% peptone water and plated in duplicate on Brain Heart Infusion Agar (BHIA) (Difco) plates using a Spiral Plater (Model D, Spiral Systems, Bethesda, MD). All plates were incubated for 24 h at 37°C and enumerated. It was checked that extended incubation did not increase count significantly. The pH of 0-h samples was measured using a pH meter (Model 601A, Orion) to ensure the initial pH had been sustained.

UHT milk was purchased from a local supermarket and prechilled to 5°C. The milk was aseptically transferred to presterilized 250-ml Erlenmeyer flasks and inoculated to achieve an initial level of 10<sup>3</sup> cfu/ml. All flasks were incubated at 5°C without agitation, with five replicate cultures being examined for each pre-inoculation temperature. During sampling, flasks were kept in an ice water bath and swirled to attain a homogeneous sample. Samples were removed, diluted, plated, incubated, and counted as described above.

Canned dog food

'All-meat' canned dog food was used as a model for cooked meat products. Three varieties (all beef, beef and liver, and meat and gravy) in 4-oz cans were purchased at a local supermarket and prechilled to 5°C along with sterile 500-ml beakers. The contents of the cans were transferred aseptically to individual beaekrs. Pre-inocula grown at 19 or 37°C were diluted and used to inoculate the samples to a target level of 10<sup>3</sup> cfu/g. The final dilution of the inoculum was made using diluent (9.9 ml) containing approximately 0.1 ml of a green food dye (Durkee, Wayne, NJ). The meat samples were blended until the dye was uniformly spread throughout the sample. All samples were incubated at 5°C. At least three replicate samples were studied for each pre-inoculation temperature and product variety combination. Periodically, duplicate 1.0-g portions of each sample were transferred to screw cap tubes containing 9.0 ml of sterile 0.1% peptone water. After agitating to disperse the meat in the diluent, appropriate dilutions were made, and 0.2-ml portions were distributed evenly across the surface of prepoured BHIA plates using a sterile glass spreader. All plates were incubated for 24 h at 37°C and enumerated. Again, it was determined that extended incubation did not increase counts significantly.

TABLE I

Equations for Gompertz function and derived growth kinetics values

## Gompertz's function:

 $L(t) = A + Ce^{-e^{B(t-M)}}$ 

where:

L(t) = Log count of bacteria at time (in hours) t (Log(cfu/ml)).

A = Asymptotic log count of bacteria as time decreases indefinitely (i.e., initial level of bacteria) (Log(cfu/ml)).

C = Asymptotic amount of growth that occurs as t increases indefinitely (i.e., number of log cycles of growth) (Log(cfu/ml)).

M = Time at which the absolute growth rate is maximal (h).

 $B = \text{Relative growth rate at } M \left( \frac{\text{Log(cfu/ml)}}{h} \right).$ 

Derived growth kinetics equations:

Exponential growth rate (EGR) = BC/e ((Log(cfu/ml))/h)

Generation time (GT) = (Log(2))e/BC (h)

Lag phase duration (LPD) = M - (1/B) (h)

Maximum population density (MPD) = A + C (Log(cfu/ml))

## Raw ground beef

Ground beef was purchased at a local supermarket, and 100-g portions were transferred to sterile 500-ml beakers. The samples were then inoculated and incubated as described above for canned dog food. Five replicate samples were examined for each pre-inoculation temperature. Periodically, duplicate 1.0-g portions of each sample were transferred to screw cap tubes containing 9.0 ml of 0.1% sterile peptone water. After mixing thoroughly and diluting, 0.2 ml of each sample was spread evenly across prepoured plates of Modified Vogel Johnson Agar (MVJ) (Buchanan et al., 1987) containing 200  $\mu$ g/ml sodium arsenite. This extremely selective medium took advantage of the high level of arsenite resistance inherent in strain Scott A. Preliminary studies indicated that this medium could quantitatively recover the organism from meat samples. This proved a very convenient system for following the growth of *L. monocytogenes* in raw meat since the test strain was essentially the only organism that grew on the MVJ + arsenite plates. All plates were incubated for 48–72 h at 37 °C and enumerated. The pH of meat samples was measured at 216 h.

# Irradiation-sterilized, raw ground beef

Ground beef purchased locally was divided into 100-g portions, vacuum packed in individual low permeability plastic bags (All-Vak #13, International Kenfield Distributing Co., Rosemont, IL), and then frozen and maintained at  $-70\,^{\circ}$ C before and during irradiation. The frozen samples were irradiated with 48.5 kGy of gamma irradiation using a Cs<sup>137</sup> source. After radiation sterilization, the meat samples were equilibrated to 5°C. The samples were then transferred aseptically to sterile, prechilled beakers and inoculated, incubated, sampled, and counted as described above for the canned dog food samples.

#### Growth curves

Where appropriate, growth curves were generated using the Gompertz function (Table I) (Buchanan et al., 1989). The four Gompertz parameters were used subsequently to calculate exponential growth rates (EGR), generation times (GT), lag phase durations (LPD), and maximum population densities (MPD).

## Results

L. monocytogenes was initially grown at various temperatures (5–42°C) to determine the effect that incubation temperature of the inoculum had on the subsequent kinetics of aerobic and anaerobic growth at 5°C in TPB (Table II). Temperature history of the inoculum had relatively little effect on either EGR, GT, or MPD, whereas more distinct differences in LPDs as a function of temperature history were observed in both the aerobic and anaerobic cultures. Aerobically, the LPD was increased 10-20 h when the inoculum was grown at  $\geq 37$ °C, as compared to the lower incubation temperatures. This effect appeared to be more pronounced with the anaerobic cultures, with an approximate 2-fold increase in

TABLE II

Effect of initially culturing *Listeria monocytogenes* Scott A at various temperatures on its subsequent growth kinetics at 5°C in tryptose phosphate broth. <sup>a</sup> (For explanation of abbreviations, see Table I)

Pre- incubation temperature		Gompertz values				LPD	EGR	GT	MPD
						(h)	(Log(cfu	(h)	(Log(cfu
			C D		<b>M</b>		/ml)/h)		/ml))
(°C)	n	A	C	В	IVI	4 .			
Aerobio	cultur	es							
5	2	2.0	7.4	0.008	159.8	36.8	0.023	13.6	9.5
		(0.0)	(0.2)	(0.001)	(4.6)	(12.0)	(0.001)	(0.5)	(0.2)
10	7	3.4	6.1	0.013	125.9	39.3	0.029	11.7	9.5
		(0.6)	(0.6)	(0.006)	(17.9)	(18.0)	(0.013)	(3.4)	(0.4)
13	2	3.5	6.1	0.013	104.2	29.1	0.030	10.1	9.6
		(0.0)	(0.3)	(0.001)	(5.7)	(3.0)	(0.002)	(0.7)	(0.3)
19	5	3.7	6.1	0.017	95.9	36.7	0.038	8.0	9.7
		(0.1)	(0.2)	(0.003)	(10.4)	(3.7)	(0.005)	(1.1)	(0.1)
28	2	3.5	6.2	0.014	108.2	36.7	0.032	9.5	9.7
		(0.1)	(0.1)	(0.001)	(5.1)	(8.7)	(0.001)	(0.4)	(0.0)
37	6	3.8	6.1	0.013	128.3	50.6	0.030	10.5	9.8
		(0.2)	(0.2)	(0.002)	(11.7)	(15.6)	(0.005)	(1.8)	(0.2)
42	2	3.7	6.3	0.013	125.9	49.6	0.031	10.0	10.0
		(0.1)	(0.2)	(0.000)	(4.0)	(4.0)	(0.001)	(0.3)	(0.1)
Anaero	bic cul	tures							:
5	2	2.0	7.5	0.010	132.2	27.4	0.027	11.4	9.6
		(0.1)	(0.1)	(0.001)	(10.0)	(4.6)	(0.002)	(0.7)	(0.1)
10	5	2.6	6.9	0.009	135.4	27.8	0.023	12.9	9.5
		(1.1)	(1.5)	(0.001)	(8.8)	(8.3)	(0.002)	(1.1)	(0.4)
13	2	3.6	5.9	0.015	89.4	24.7	0.034	9.0	9.6
		(0.0)	(0.1)	(0.000)	(1.6)	(0.1)	(0.001)	(0.1)	(0.1)
19	2	3.7	5.9	0.016	109.4	49.1	0.036	8.5	9.6
		(0.0)	(0.1)	(0.001)	(1.6)	(5.9)	(0.002)	(0.5)	(0.1)
28	2	3.7	6.2	0.014	121.4	51.7	0.033	9.2	9.9
	_	(0.0)	(0.0)	(0.000)	(11.7)	(10.8)	(0.001)	(0.1)	(0.0)
37	6	3.7	6.0	0.015	121.0	50.0	0.032	9.6	9.7
	. •	(0.2)	(0.1)	(0.002)	(12.6)	(8.5)	(0.005)	(1.7)	(0.1)
42	2	3.6	6.2	0.015	112.3	45.4	0.034	8.8	9.9
		(0.0)	(0.0)	(0.000)	(2.8)	(3.1)	(0.000)	(0.1)	(0.1)

<sup>&</sup>lt;sup>a</sup> Values are means of *n* replicate cultures. Values in parentheses are standard deviations.

LPD being observed with the higher inoculum cultivation temperature. The effect was also more pronounced in that increased LPD's were observed at pre-inoculation temperatures as low as 19°C in the anaerobic cultures, compared to the 37°C cutoff in the aerobic cultures.

A similar pattern was observed when UHT milk was inoculated with L. monocytogenes cells grown at 19 or 37°C (Fig. 1). An approximate 3-fold increase in the

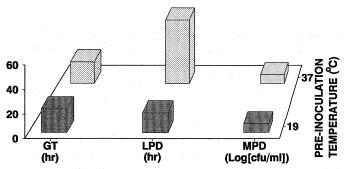


Fig. 1. Effect of initially culturing *L. monocytogenes* Scott A aerobically at 19 and 37°C on its subsequent growth at 5°C in UHT-pasteurized milk. GT = generation time, LPD = lag phase duration, MPD = maximum population density. Average of five determinations.

mean LPD (16.2  $\pm$  12.1 vs. 51.9  $\pm$  18.7 h) was observed with the higher pre-inoculation temperature, whereas the mean GTs (19.3  $\pm$  4.0 vs. 17.7  $\pm$  3.3 h) and MPDs (7.4  $\pm$  0.1 vs. 7.6  $\pm$  0.2 log(cfu/ml)) of the cultures were similar.

Commercially available 'all meat' dog food was used as a model for cooked meat products. The 4-oz cans of this type of product proved to be a convenient test

TABLE III

Effect of initially culturing *Listeria monocytogenes* Scott A aerobically at 19 vs. 37°C on its subsequent growth kinetics at 5°C in canned dog food <sup>a</sup> (For explanation of abbreviations, see Table I)

Product	Pre-	Gompertz va	lues		LPD (h)	EGR (Log(cfu /ml)/h)	GT (h)	MPD (Log(cfu /ml))
variety b	incubation							
	temperatur	e 8 % a 8						
	(°C)	$\overline{A}$ $C$	В	M				
1	19	1.6 6.7	0.0063	260.9	61.2	0.015	25.0	8.3
		(0.4) (0.3)	(0.0014)	(63.5)	(5.8)	(0.004)	(8.0)	(0.3)
	37	2.6 5.5	0.0059	249.6	54.3	0.012	28.9	8.2
		(0.1) $(0.2)$	(0.0013)	(22.5)	(7.7)	(0.003)	(4.4)	(0.2)
2	19	1.7 6.8	0.0068	207.3	52.0	0.017	18.7	8.5
		(0.2) $(0.2)$	(0.0007)	(19.3)	(2.3)	(0.002)	(2.0)	(0.1)
	37	2.7 5.6	0.0108	166.9	73.7	0.022	13.6	8.3
		(0.1) (0.1)	(0.0004)	(9.2)	(10.7)	(0.001)	(0.4)	(0.1)
3	19	1.7 6.5	0.0051	254.3	46.0	0.012	26.4	8.2
	er gjalani.	(0.2) $(0.2)$	(0.0007)	(10.2)	(16.9)	(0.002)	(2.8)	(0.3)
	37	2.7 6.3	0.0054	274.2	88.8	0.012	24.1	8.9
		(0.1) (0.2)	(0.0003)	(22.6)	(31.4)	(0.001)	(0.5)	(0.3)

<sup>&</sup>lt;sup>a</sup> Values are means of at least three independent determinations. Values in parentheses are standard deviations.

b 1 = 'all beef'; 2 = 'beef and liver'; 3 = 'meat and gravy'.

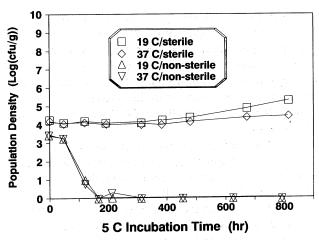


Fig. 2. Effect of initially culturing L. monocytogenes Scott A aerobically at 19 and 37°C on its subsequent survival at 5°C in raw ('non-sterile') ground beef and gamma irradiated ('sterile') raw ground beef. Average of three determinations.

system, providing an adequately sterilized, reasonably homogeneous meat-based product. Three different product varieties were evaluated in conjunction with pre-inoculation temperatures of 19 and 37 °C (Table III). The MPDs were roughly

TABLE IV

Taxonomic characteristics of isolates from ground beef that possessed activity against Listeria monocytogenes Scott A

Shape	Medium length rods		
Gram stain	+		
Motility	n samu <u>ta</u> n na masa na Tangan na masa		
Catalase			
Growth at 12°C	ana s <del>t</del> a ntak i salifik ti fa sejis		
Tellurite reduction	to th <del>a</del> sois a display of a soit.	eng endido	
H <sub>2</sub> S production			
Carbohydrate	3 Days	7 Days	<u>gar are its</u> e sikur
	28°C 12°C	28°C	12°C
Glucose	+ - + +	+	
Sucrose	ing gerger engle + 1	: ; ;* <del>+</del> ;	, <del>+</del>
Maltose		+ <del>+</del> ,,	+
Mannose	1	+	
Lactose	+	± .	
Mannitol	+ 1111	+	+
Xylose		±	<del>-</del>
Ribose		+	+ ,
Rhamnose	joon ju <b>±</b> a jo <sup>li</sup> a de la la <del>z</del> ije	<del></del> -	, <del>-</del> :
Arabinose	taka ±jaa ja njin aj≂je	- <del>-</del> -	: <del>-</del>
Melibiose	+	+	• • • <u>•</u> • • • • • • • • • • • • • • •

equivalent regardless of product type or pre-inoculation temperature. EGR/GT was unaffected by pre-inoculation temperatures in two of the product varieties (No. 1 and No. 3), but an approximate 30% decrease in GT was observed with the 37°C/variety No. 2 cultures. The effect of pre-inoculation temperature on LPD was also product-dependent. An approximate 50% increase in LPD was observed with the higher pre-inoculation temperature in variety No. 2, and a 2-fold increase occurred with variety No. 3. However, the LPD of variety No. 1 was unaffected by pre-inoculation temperature.

Raw ground beef sterilized by gamma irradiation did not support significant growth at 5°C of *L. monocytogenes* regardless of pre-inoculation temperature (Fig. 2); however, the microorganism did survive for extended periods. When unsterilized raw ground beef was inoculated with cells initially incubated at 19 and 37°C, the microorganism died off at a relatively rapid rate. The pH of the meat at 216 h, the time when inactivation was complete was 7.2–7.4. Representative members of the meat's microflora were isolated and screened for activity against *L. monocytogenes* using BHIA plates seeded with a lawn of *L. monocytogenes* Scott A. Two isolates produced distinct zones of inhibition. Both isolates had similar taxonomic characteristics and were tentatively classified (Sneath et al., 1986) as *Lactobacillus plantarum*, based on the criteria list in Table IV.

#### Discussion

The lag phase is an adjustment period during which bacterial cells modify themselves and their surroundings in order to take advantage of a new environment and initiate exponential growth. This implies that the smaller the degree of difference between the new and old environments, the smaller the duration of the lag phase. The results of the current study on the effects of temperature history on the 5°C growth kinetics support this in a general manner; however, they indicate that the response does not follow a continuum. Instead, there were critical pre-inoculation temperatures above which extended LPD's were observed (Table II). This suggests that the phenomenon involves an underlying on/off physiological process and not a gradual increase or decrease in a cellular component(s). The differential response between aerobic and anaerobic cultures (Table II) suggests that components involved in the microorganism's oxidative metabolite processes play a role in determining the impact of temperature history on subsequent low temperature growth. Identification of the factors underlying the altered sensitivity of anaerobic cultures to temperature history will require additional research.

Demonstration that incubation temperature of the inoculum can influence the subsequent low temperature growth of *L. monocytogenes* in UHT milk (Fig. 1) and model products for cooked meats (Table III) indicates that temperature history may have a small but measurable impact on the growth of the microorganism in refrigerated foods. The results indicate that cells of *L. monocytogenes* adapted to a cooler environment (e.g., chill rooms, refrigerated ingredients, etc) would initiate growth in a refrigerated food somewhat sooner than cells coming from a warm

environment (e.g., food handlers). The differing response among canned dog food varieties (Table II) indicates further that there may be nutritional/formulation factors that influence the response of the microorganism to temperature history. The ingredient labels on these products were not detailed enough to allow comparison of the formulations of the varieties, and further investigations will be needed to identify the cause of the differential responses observed among the product varieties.

The increase in L. monocytogenes levels of less than a log cycle in irradiationsterilized, raw ground beef after more than a month of storage at 5°C, regardless of pre-inoculation temperatures of 19 and 37°C, does not support the hypothesis of Grau and Vanderline (1988) that the lack of growth of the pathogen in refrigerated meat reported by various investigators is due to the use of elevated temperatures for culturing inocula. The small differences noted between the cultures initially incubated at 19 and 37°C toward the end of the 5°C storage period were too small to be distinguished from the normal variation associated with plating microbial samples. It is possible that the levels of L. monocytogenes in the irradiation-sterilized meat would have continued to increase if the incubation period were extended further; however, the time frames are substantially different from those of Grau and Vanderlinde (1988) who reported that the levels of L. monocytogenes increased from 10<sup>3</sup> to 10<sup>7</sup> cfu/cm<sup>2</sup> within 16 and 21 days of incubation at 5.3°C on the fat and lean surfaces of beef strips, respectively. Similarly, Gill and Reichel (1989) found that L. monocytogenes reached levels of 107 cfu/sample within one month on beef strips incubated at 5°C. It is worth noting that these time frames are similar to those observed with cooked meat products (i.e., canned dog food) in the current study.

The use of irradiation-sterilized meat eliminated the possibility that the lack of growth in raw ground beef was due to a competing microflora. This suggests that the failure of L. monocytogenes to grow was due to either an inhibitory condition or component in raw ground beef that is eliminated upon cooking. It is possible that the differences between the current study and that of Grau and Vanderlinde (1988) and Gill and Reichel (1989) were due to different strains of L. monocytogenes being employed; however, this seems unlikely since a variety of strains have been used by other investigators (Kahn et al. 1972, 1973, 1975; Gouet et al., 1978; Buchanan et al., 1987; Johnson et al., 1988a,b; Shelef, 1989; Kaya and Schmidt, 1989) who have reported no growth in raw meat. An explanation worth investigating is that the observed differences among the reports were due to the physical form of the meat. The investigators reporting a lack of growth at refrigerated temperatures used raw comminuted meats whereas those reporting growth employed meat strips. This suggests that some attribute of the surface of meat favors the growth of the organism or inactivates the hypothesized inhibitory condition/component. One possibility is that comminution and dispersal of the inoculum throughout the meat sample results in high CO<sub>2</sub> microenvironments. Gill and Reichel (1989) reported that, while L. monocytogenes grew at temperatures as low as 0°C on vacuumpackaged beef strips, the organism did not grow below 10°C when incubated in an elevated CO<sub>2</sub> atmosphere.

In addition to establishing that the lack of growth was not due to a competing microflora, the use of irradiation-sterilized ground beef helped demonstrate that the inactivation of L. monocytogenes in untreated ground beef was a function of the microflora. The relatively rapid rate of inactivation, in conjunction with the neutral pH of the mean samples after 200 h of incubation, suggest the presence of competing microorganisms capable of producing an antimicrobial agent(s) that affects the pathogen. Gouet et al. (1978) reported that the level of L. monocytogenes decreased when it was co-cultured at 8°C with L. plantarum in sterile ground beef. A number of bacteriocins produced by various bacterial species have been reported to have activity against L. monocytogenes (Hoover et al., 1988; Pucci et al., 1988; Bhunia et al., 1988; Benkerroum and Sandine, 1988; Carminati et al., 1989; Chung et al., 1989; Ortel, 1989). The current isolation of a L. plantarum strain with activity against L. monocytogenes as a major component of the microflora of ground beef suggests that the lactic acid bacterium may have played a role in the inactivation of the pathogen. This suggests further that utilization of selected cultures with activity against L. monocytogenes has potential as a means of controlling the pathogen in mean products. Investigations to characterize the activity of the L. plantarum isolates are currently underway.

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